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Integration of fluidic channels and total internal reflection excitation with supercritical angle fluorescence detection

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Abstract. In many diagnostic applications, there is a growing demand for small cost effective portable devices that enable fast on-site analysis. For this target, we are developing an integrated approach of a previously reported surface-sensitive measurement system based on total internal reflection excitation and supercritical angle fluorescence detection. The integrated measurement platform includes a polystyrene substrate on which the fluidic channels for sample transport, waveguides for fluorescence excitation and the readout optics are fabricated. In addition, thin layers with dielectric properties differing from those of polystyrene may be needed on the fluidic channels for optimal sample transport or biomolecule immobilisation. Here, special attention is paid to the influence of the dielectric environment near the solid-liquid interface on the fluorescence distribution and to the design of the readout optics. The first version of the designed readout optics of the integrated measurement platform is presented.

1 Introduction

Optical biosensors have attracted considerable research interest during the past several decades due to their versatile implementation possibilities, potential selectivity and immunity to electromagnetic interferences. Especially, fluorescence based detection has been considered beneficial with respect to sensitivity and achievable limits of detection. In many diagnostic applications, there is also a need for fast on-site analysis requiring small, cost effective portable systems. In this paper, a step towards this direction is taken by designing a low-cost polymeric substrate that enables both the fabrication of the fluidic channels for optimal sample transport and immobilisation of biomolecules combined with waveguide based total internal reflection (TIR) excitation and optical readout utilizing supercritical angle fluorescence detection (SAF) [1]. In the proposed approach, the SAF emitted by the molecules bound to the bottom of the fluidic channel generate a specific radiation distribution that is defined by the dielectric properties of the sample liquid and the substrate as well as by the distance of the fluorescing molecule from the bottom of the fluid channel [2-3]. Here, different dielectric environments near the bottom of the fluidic channel are studied in relation to the SAF fluorescence emission distribution that is then used as an input for designing the readout optics of the integrated measurement system described below.

2 Materials and methods

2.1. SAF emission

The SAF emission distributions are calculated for a randomly oriented dipole located at different distances from the solid-liquid interface at the bottom of the fluidic channel following the presentation of Enderlein et al. [3]. The results are calculated for two different liquid samples, water and milk, with the emission wavelength of the fluorescence being 670 nm (Figure 1).

Table 1. Optical parameters used in the simulations.

	Polystyrene	Al_2O_3	Water	Milk
Refractive index	1.58645	1.76581	1.33	1.344

2.1. Optics

In the integrated measurement platform (Figure 2), the optical readout device combines the TIR based excitation channel [1] and SAF based parabolic readout optics as presented in ref. [2]. In the excitation light guide, only the angles above the critical angle are allowed and can be controlled while coupling the light into the light guide.

The fluorescent emission is collected by a parabolic collimator lens, which has the focal point at the SAF emission point. A cylindrical aperture blocker and high-

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pass filters limit the angular and spectral band before the light is focused to a detector by a plano-convex lens.

The integration sets some restrictions to the size and, thus, to the collection efficiency of the parabolic concentrator. First, the minimum diameter of the parabolic lens at the narrow end is limited by the space reservation of the fluidic channel and light guide. Secondly, the thickness of the whole system is limited by the thickness reservation of the excitation channel and the system thickness in general. Especially the overlap of the relatively wide excitation channel used in this first prototype and the parabolic concentrator reduces the collection efficiency.

Polystyrene (PS) was selected as the material for the optical readout device. Its high refractive index, when compared to other plastics, together with the possibility to use injection moulding, were the main reasons for the material selection.

The readout device was designed using the Optics Studio non-sequential design tool. The simulated angular distributions in polystyrene were modelled as a pointwise defined radial source.

3 Results and discussion

3.1 SAF emission distributions

Figure 1 shows the calculated fluorescence distributions expressed as normalized radiated power as a function of the emission angle in the polystyrene substrate. The results are shown for water and milk as the sample matrices with and without an Al_2O_3 coating of 5 nm in thickness on the bottom of the fluid channel. Due to their fairly similar refractive indices, the curves for water and milk follow closely each other, but the 5 nm thick Al_2O_3 coating has a significant effect especially at high emission angles. The highest power is obtained at the critical angle.

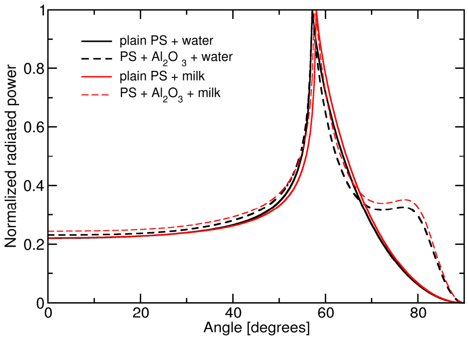

Fig. 1. Fluorescence distributions: the normalized radiated power as a function of the emission angle in the polystyrene substrate for water and milk with and without 5 nm Al_2O_3 coating on the bottom of the fluid channel.

3.2 Optics

The optical system design and spatial irradiance distribution of the reflected rays are shown in Figure 2.

The fluidic channel locates on the light guide on the surface opposite to the readout optics although not shown in the figure. The diameter of the collection optics is 12.5 mm and the thickness of the parabolic concentrator 3.5 mm. The parabolic concentrator can collect SAF angles at about 57.5 degrees and above. The irradiance profile visualises how the irradiance increases towards the edges of the spatial filter that is towards the critical angle. When reflection losses are excluded, the total SAF collection efficiency is about 30% and it is mainly limited by the overlap of the excitation channel and parabolic concentrator. The system will be injection moulded and it is assumed that some modifications has to be made in order to optimise the system for the specific applications.

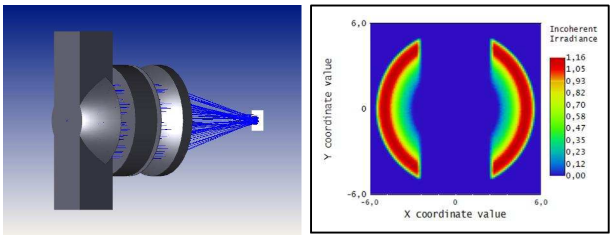

Fig. 2. The measurement platform showing the (vertical) light guide and the readout optics with the parabolic lens (left) and the relative irradiance distribution of the reflected rays at the spatial filter to be focused to the detector (right).

3 Conclusions

It was shown that even very thin additional layers on the fluidic channels can have a significant influence on the SAF emission distribution that need to be taken into account when designing the readout optics.

The readout optics simulations show that the SAF angles higher than 57.5 degrees can be measured with the integrated measurement platform and that the overall SAF collection efficiency is about 30% for the relatively wide excitation channel used in the current design. The collection efficiency is mainly limited by the overlapping parabolic lens and excitation channel, in addition to the inherent property of the SAF detection rejecting the emission in the angles below the total internal reflection.

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